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(54) Title: A PROCESS FOR THE PREPARATION OF STEM CELLS FROM HUMAN MUSCLE TISSUE AND ADIPOSE TISSUE, AND STEM CELLS OBTAINABLE BY THIS PROCESS

(57) Abstract: The invention relates to a process for the preparation of the human stem cells from muscle tissue or adipose tissue. The process provides for the incubation of cells obtained from a sample of muscle tissue or adipose tissue in a medium comprising BSA, Bfgf, EGF, VEGF, LIF, heparin and usual inorganic salts, natural acids and vitamins necessary for the growth of mamalian cells. The invention relates also to the human muscle stem cells (hMSC) and human adipose tissue cells (hFSC) obtainable by this process.

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AMENDED CLAIMS

[received by the International Bureau on 14 July 2004 (14.07.04);
original claims 1 - 14 amended (4 pages)]

1. A process for the preparation of human stem cells from a sample of human adipose or muscle tissue, said human stem cells being capable of differentiating into nerve cells, vascular cells and bone cells, the process comprising the steps of:

a) preparing a cell suspension from a sample of human adipose or muscle tissue;

b) recovering the cells from the cell suspension; and

c) incubating these cells in a medium comprising BSA, bFGF, EGF, VEGF, LIF, heparin and usual inorganic salts, natural amino acids and vitamins necessary for the growth of mammalian cells.

2. A process according to claim 2, wherein the medium is DMEM/F12 supplemented with: from 0.4% to 0.8% of BSA, from 5 to 20 ng/ml of bFGF, from 10 to 40 ng/ml of EGF, from 2.5 to 10 ng/ml of VEGF, from 5 to 20 ng/ml of LIF, from 1 to 20 μg/ml of heparin, from 1.8 to 3 mg/ml of glucose, from 2 to 2.5 mg/ml of $NaHCO_3$, from $2.5x10^{-3}$ to $7.5x10^{-3}$ M of Hepes, from 50 to 200 μg/ml of apotransferrin, from 10 to 30 μg/ml of insulin, from $3x10^{-4}$ to $7x10^{-4}$ M of putrescine, from $4x10^{-8}$ to $8x10^{-8}$ M of selenium, from $1x10^{-8}$ to $3x10^{-8}$ M of progesterone.

3. A process according to claim 1 or 2, wherein the tissue sample is a human skeletal muscle sample and the stem cells are human muscle stem cells (hMSC).

4. A process according to claim 3, wherein step a) comprises the digestion of the skeletal muscle sample with trypsin.

5. A process according to claim 3 or 4, wherein step c) comprises:

c_1) resuspending the cells recovered from the cell suspension of step a) in the growth medium as defined in claim 1 or 2;

c_2) incubating the cell suspension obtained in the previous step inside a container for cell cultures, which has previously been treated with type I collagen, for from 18 to 24 hours at a temperature of approximately 37°C and in a 5% CO_2 atmosphere;

c_3) removing the growth medium from the container and replacing it with an identical freshly prepared growth medium; and

c_4) incubating for a further 48 to 72 hours, thereby obtaining the formation of small roundish cells adhering to the walls of the container, the small adherent roundish cells being human muscle stem cells (hMSC).

6. Human muscle stem cells (hMSC) capable of differentiating into nerve cells, vascular cells and bone cells, obtainable by a process according to any one of claims 3 to 5.

7. A process according to claim 1 or 2, wherein the tissue sample is a human adipose tissue sample and the stem cells are human adipose tissue stem cells (hFSC).

8. A process according to claim 7, wherein said step c) comprises:

c_1) resuspending the cells recovered from the cell suspension of step a) in the growth medium as defined in claim 1 or 2;

c_2) incubating the cell suspension obtained in the previous step inside a container for cell cultures, which has previously been treated with type I collagen, for from 18 to 24 hours at a temperature of approximately 37°C and in a 5% CO_2 atmosphere;

c_3) recovering the cells not adhering to the walls of the container and resuspending them in freshly prepared growth medium as defined in claim 1 or 2;

c_4) placing the cell suspension obtained in the previous step in a second container for cell cultures which has not previously been treated with collagen and cultivating the cells therein for from 7 to 10 days, thus obtaining the formation of floating cell aggregates, the cells of these aggregates being human adipose tissue stem cells (hFSC).

9. A process according to claim 8, wherein steps c_2) and c_3) are repeated twice more.

10. Human adipose tissue stem cells (hFSC) capable of differentiating into nerve cells, vascular cells and bone cells, obtainable by a process according to any one of claims 7 to 9.

11. Stem cells according to claim 6 or 10 for use in the regeneration of tissue selected from the group consisting of bone tissue, cartilaginous tissue, endothelial tissue, smooth muscle tissue, striated muscle tissue and nerve tissue.

12. Stem cells according to claim 6 or 10 for use in the treatment of ischaemic tissue, in the repair of vascular damage, in the cell treatment of myocardial infarct, in co-transplantation with other stem cells or tissues, in the production of growth and/or trophic factors, in the production of hormones, in tissue bioengineering, in the regeneration of peripheral nerves, in the treatment of multiple sclerosis, in the treatment of myocardial infarct, in the treatment of Alzheimer's disease or in the treatment of Parkinson's disease.

13. Use of stem cells according to claim 6 or 10 for the preparation of a medicament for the regeneration of tissue selected from the group consisting of bone tissue, cartilaginous tissue, endothelial tissue, smooth muscle tissue, striated muscle tissue and nerve tissue.

14. Use of stem cells according to claim 6 or 10 for the preparation of a medicament for the treatment of ischaemic tissue, the repair of vascular damage, the cell treatment of myocardial infarct, co-transplantation with other stem cells or tissues, the regeneration of peripheral nerves, the treatment of multiple sclerosis, the treatment of myocardial infarct, the treatment of Alzheimer's disease or the treatment of Parkinson's disease.